



# Tissue mechanics regulates form, function, and dysfunction

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Morphogenesis encompasses the developmental processes that reorganize groups of cells into functional tissues and organs. The spatiotemporal patterning of individual cell behaviors is influenced by how cells perceive and respond to mechanical forces, and determines final tissue architecture. Here, we review recent work examining the physical mechanisms of tissue morphogenesis in vertebrate and invertebrate models, discuss how epithelial cells employ contractility to induce global changes that lead to tissue folding, and describe how tissue form itself regulates cell behavior. We then highlight novel tools to recapitulate these processes in engineered tissues.

## Addresses

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## Introduction

Morphogenesis determines the unique shape and correct positioning of tissues and organs in the body. Just as all cells come from cells (*'omnis cellula e cellula'*) [1], all tissues come from cells that contain essentially the same genetic information. Many of the signaling pathways that control organ morphogenesis are conserved across species [2], and common changes in cell adhesion, cell shape, and cell migration drive morphological changes on a tissue scale. Nonetheless, every tissue exhibits a distinct architecture and function, which indicates that cells integrate information from signaling networks and mechanical cues in a context-dependent manner to determine the physical output of gene expression [3,4].

The spatiotemporal control of morphogenetic processes accommodates and is driven by surface area and volume

constraints to give rise to various tissue architectures: from arborized networks of blood vessels, neurons, and bronchial tubes to vilified epithelial sheets. In order to meet mass-transport requirements, most animals employ a network of interconnected epithelial tubes with barrier and secretory functions [5]. For instance, the human vascular network enables about five liters of blood to be delivered to tissues each minute [6], while the arborized structure of the lungs maximizes the surface area for gas exchange at the alveolar tips to enable the oxygenation of blood. How groups of epithelial cells form polarized sheets that buckle and bend in response to mechanical and biochemical cues, and thus acquire various shapes and functions, remains mostly a mystery. It is well appreciated, however, that the generation and maintenance of proper tissue architecture is required for homeostasis whereas its loss is a prerequisite for disease [3].

Studies of model organisms and cultured tissues have provided key insights into how mechanical forces generated at the cellular level are integrated with biochemical cues to convert gene expression patterns into sophisticated tissue structures in a context-dependent manner. Most of our understanding of morphogenetic processes emanates from well-defined invertebrate models because of widely available genetic and molecular tools. A well-studied example is ventral furrow formation in *Drosophila*, during which the tension generated by actomyosin contractility across the apical surface of a sheet leads to apical constriction and localized tissue folding [7–9]. This requires dynamic changes in actomyosin contractility at the molecular level to be transmitted across larger length scales through junctional domains between cells in the tissue sheet [10].

Development is choreographed such that tissue structure can be tuned in response to microenvironmental factors. The interactions between the cells that constitute a tissue and their surrounding extracellular matrix (ECM) can guide cellular behavior and changes in tissue morphology. According to the principle of dynamic reciprocity, cells communicate with the ECM through the transport of growth factors or through direct contact with membrane-associated components, and these interactions evolve over time [11]. This crosstalk has been examined extensively in the context of the mammary gland, which can undergo cycles of development, differentiation, and apoptosis in order to accommodate the temporary need to produce and deliver milk [12]. The regulation of ECM remodeling in morphogenesis has revealed that the loss of

proper tissue architecture underlies malignant transformation, while reconstitution of normal tissue architecture through the restoration of healthy cell–ECM communication overrides genetic abnormalities [3,4,13–15].

Disrupting the force-generation and transmission machinery leads to aberrant tissue morphologies that underlie many congenital diseases such as defects of neural tube closure, pulmonary hypoplasia, and abnormal alveolar structures [16]. Morphogenesis of diseased tissues relies on the same signaling pathways that guide healthy development. Thus in a way, acquired diseases such as cancers are errors of development, as Virchow asserted, since ‘tumors appear by the same law which regulated embryonic development’ [1].

Here, we discuss how a group of undifferentiated cells employ cytoskeletal contractility, proliferation, apoptosis, and interactions with their surrounding microenvironment to generate complex and reproducible epithelial tissue architectures. We review recent work on how long-range transmission of mechanical forces sculpts sheets of cells into their final form, and how its dysregulation leads to the disruption of healthy tissue architecture.

### Cellular contractility generates tissue folds

Many morphogenetic events that remodel epithelial sheets result from dynamic changes in cell shape. A well-known example is apical constriction, in which the apical surface of a cell shrinks due to the purse-string effect produced by actomyosin contractility [17]. This local change in cell geometry impacts global tissue morphology when contractile forces are transmitted across a sheet through cell–cell junctions, and its role has been implicated in cell ingression, cell extrusion, delamination, and wound healing [17,18].

Actomyosin contractility has been shown to underlie the initiation of epithelial buds during branching morphogenesis of the chicken lung. Localization of phosphorylated myosin light chain (pMLC) and filamentous actin (f-actin) to the apical surface of the epithelium was demonstrated to induce cellular shape changes as a result of apical constriction that precedes domain branching, and induces branch initiation (Figure 1a1). Inhibiting actomyosin contractility prevented both apical constriction and domain branching, whereas blocking proliferation had no effect on branch initiation [19].

Ventral furrow formation in *Drosophila* is driven by dynamic pulsatile actomyosin contractions [7], and the coordination of these pulses leads to collective apical constriction [20] that drives individual cell shape changes. The transmission of contractile forces relies on the coupling of cell–cell junctions to actomyosin networks [21]; recently, the use of optogenetic tools to manipulate cytoskeletal contractility with spatial specificity

demonstrated for the first time that depleting actin from the cortex arrested invagination of the ventral furrow [22]. Guglielmi *et al.* used light to modulate the levels of plasma membrane phosphoinositides, or phosphatidylinositol-4,5-bisphosphates, which regulate cortical actin polymerization, achieving spatiotemporal control over cellular contractility. These experiments demonstrated that apical constriction is necessary to both initiate and sustain invagination [22]. Since this optogenetic approach provides spatial and temporal control over apical constriction, it could be used in other developmental systems to assess the extent of force transmission required to induce tissue folding.

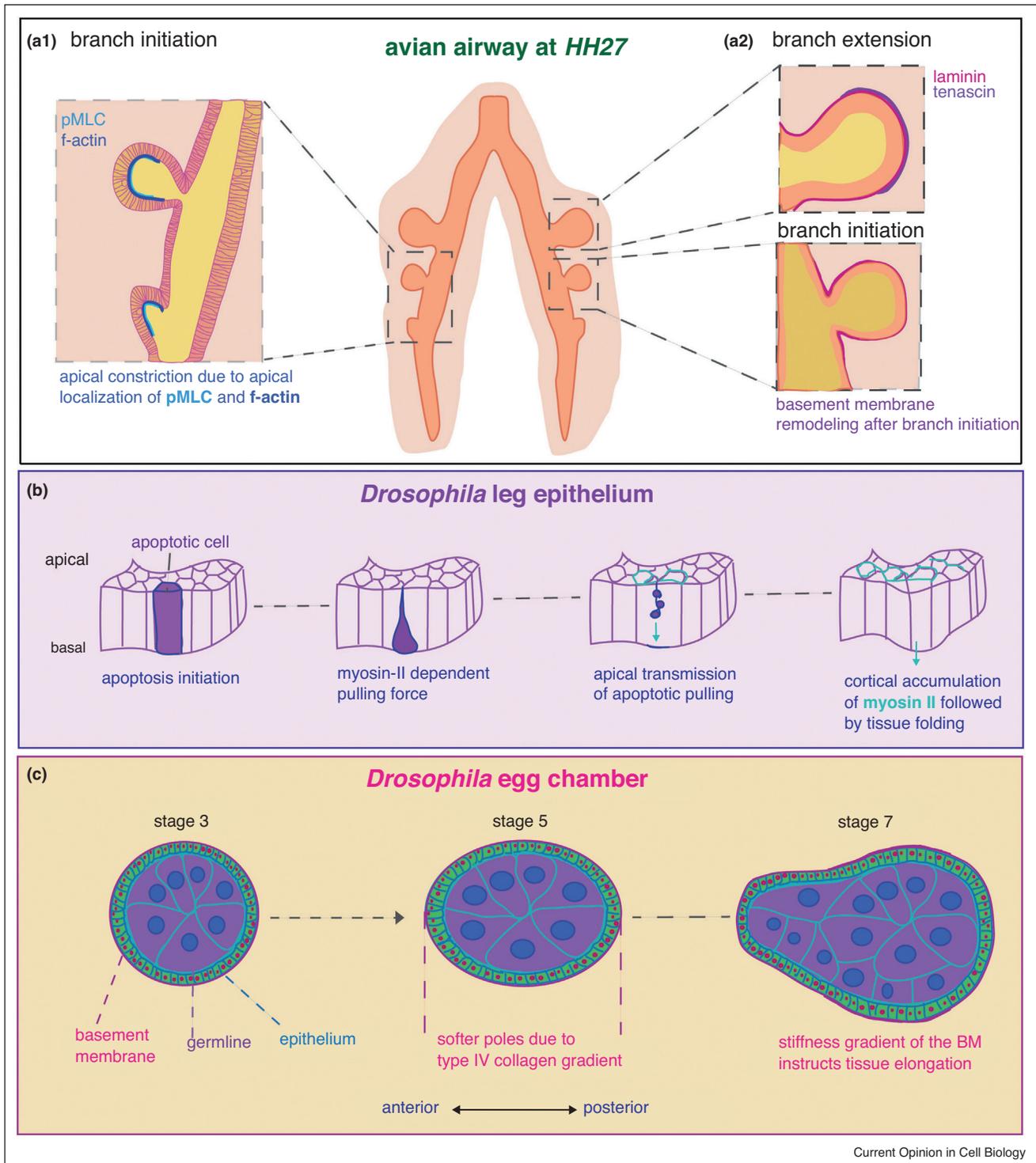
Actomyosin contractility also has an important role in providing the mechanical forces necessary to drive cytokinesis during cell division [23], and causes local tissue deformation by inducing cell-shape changes in apoptotic cells [24]. Recently, it was found that actomyosin contractility drives epithelial folding in the *Drosophila* leg by creating an apico-basally directed force in apoptotic cells. Following the initiation of apoptosis, it was observed that a cable-like myosin II structure in apoptotic cells deforms the apical surface of the epithelium through myosin II-dependent pulling (Figure 1b). This force then propagates throughout the fold domain via adherens junctions, and finally, the distribution of apoptotic events within the fold domain leads to a global redistribution of myosin II to induce epithelial folding [25\*].

Cellular contractility drives the initiation of unique tissue patterns, and it is in turn modulated by predefined spatial constraints. During gastrulation in *Drosophila*, mechanical constraints imposed by the ellipsoid shape of the embryo lead to anisotropic tension along its long axis, causing the actomyosin meshwork to be aligned in anterior–posterior direction, and leads to ventral furrow formation [26\*]. These findings point to the reciprocal nature of mechanosensing, since actomyosin contractility can drive tissue folding but results as a consequence of mechanical constraints imposed by the microenvironment.

### Reciprocal interactions between cells and their surrounding microenvironment determine final tissue architecture

Crosstalk between cells and their surrounding microenvironment dictates the various patterns of cell shape changes, proliferation, apoptosis, and rearrangement of cells within an epithelial sheet. The basement membrane (BM), a specialized type of ECM comprised mainly of laminin, collagen IV, and several large glycoproteins, separates the epithelium from its surrounding mesenchyme [27]. During branching morphogenesis of organs such as the lung, salivary gland, and mammary gland, the epithelium expands rapidly while still being enveloped within a BM [19,28,29].

Figure 1



Tissue folding arises in response to cellular contractility and physical constraints imposed by the tissue microenvironment. (a1) Domain branching in the chicken lung is preceded by apical constriction [18] and (a2) accompanied by BM remodeling. (b) In *Drosophila*, apoptotic cells pull the leg epithelium in the apicobasal direction to drive folding [24]. (c) The *Drosophila* egg chamber elongation is driven by stiffness gradients present in the BM [29].

Recent work has shown that the mechanical properties of the BM dictate organ shape. A stiffness gradient present within the BM was found to determine the aspect ratio of the *Drosophila* egg chamber (Figure 1c), causing the initially spherical structure to elongate into an ellipsoid shape [30]. It was determined that type IV collagen stiffens the BM, which in turn sculpts the egg chamber. The stiffening behavior of type IV collagen could have implications for branching morphogenesis of vertebrate tissues, including the salivary and mammary glands, since collagen IV is abundant in these BMs as well.

In murine salivary gland morphogenesis, the BM surrounding an emerging branch becomes perforated around the expanding tip, and also translocates towards the stalk to support and sculpt the extending branch. It has been suggested that the perforation of the BM is made possible by myosin-II-dependent pulling as well as protease activity [27]. Similarly, in the embryonic chicken lung, thinning of the BM accompanies branch extension, and BM remodeling persists throughout branch development. Specifically, the distribution of BM proteins tenascin C and laminin changes during branch initiation, suggesting a role for the BM in shaping the developing branch (Spurlin *et al.*, unpublished) (Figure 1a2). These findings suggest that the BM is not a static scaffold, and that communication between the epithelium and the mesenchyme patterns morphogenesis of these organs [31].

Morphogenesis of the looping structure of the murine gut also requires crosstalk between the growing epithelium and the surrounding mesenchyme. In this case, the developing smooth muscle functions as a stiff sheath in the mesenchyme that compresses the expanding epithelial tube, causing it to buckle inwards to give rise to the ridges that later form intestinal villi [32]. Local smooth muscle differentiation that impacts the mechanical properties of the mesenchyme surrounding the murine airway epithelium also guides its branching morphogenesis. The developing lung emerges from the ventral surface of the foregut endoderm, and is initially a simple epithelial tube surrounded by mesenchyme [33]. New branches emerge sequentially through domain branching followed by orthogonal and planar bifurcations at branch tips [34,35]. The mesenchyme surrounding the airway epithelium sculpts bifurcations of the extending branch through the localized differentiation of alpha-smooth muscle actin ( $\alpha$ SMA)-expressing airway smooth muscle cells (Figure 2a) [36]. A similar mechanism could underlie domain branching of the mouse lung, which is known to be pseudostratified during branch initiation (Figure 2b). These findings suggest that morphogenesis of the mouse airway and intestinal epithelia are both controlled by their surrounding mechanical microenvironments.

In addition to ECM and smooth muscle, epithelial morphogenesis can be instructed by mechanical signals from

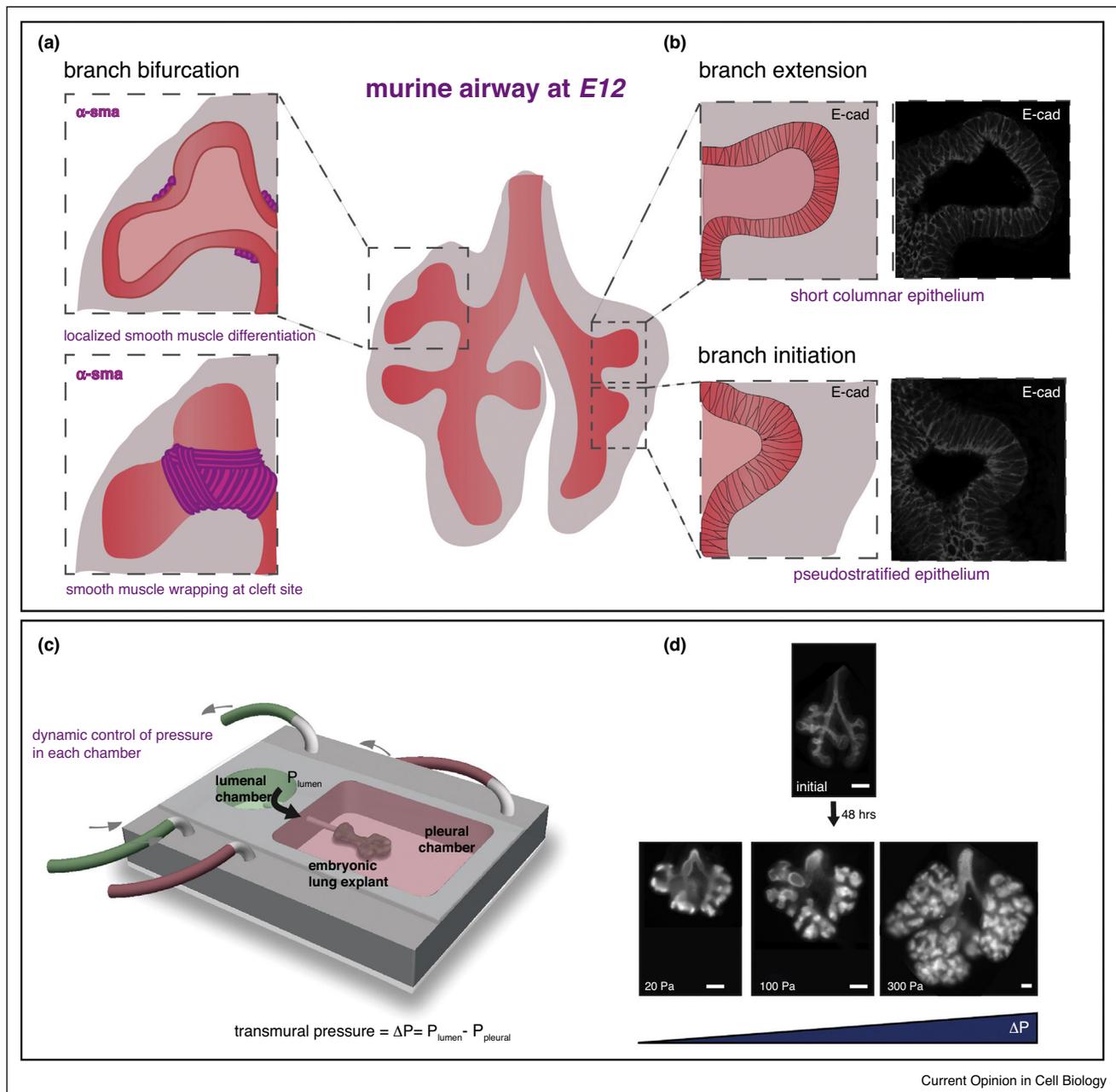
fluid pressure. Transmural pressure, which is the difference between the pressure inside and outside of an epithelial tube, was recently shown to control the rate of airway epithelial branching in the mouse lung [37]. Microfluidic chest cavities were used to culture embryonic lungs under a range of transmural pressures that represented those observed during normal development and disease (Figure 2c). In lungs cultured under low transmural pressure, few new branches formed, whereas under higher transmural pressure, the lungs developed the stereotyped branching pattern that forms *in vivo*, demonstrating that the rate of epithelial morphogenesis depends on pressure across the fetal lung (Figure 2d). Transmural pressure was also found to govern contraction of airway smooth muscle [37], which suggests that increasing the frequency of smooth muscle contraction could revert the progression of congenital diseases such as airway hypoplasia, a condition in which fetal lungs are under-branched. This demonstrates that the mechanical microenvironment facilitates crosstalk between developing or already-patterned epithelia and their surrounding tissues, and is therefore crucial for driving morphogenesis or maintaining homeostasis.

### Measuring forces in a physiologically relevant context

Although many of the cellular structures that generate and transmit force are known, and the role of the mechanical microenvironment in tissue morphogenesis is widely recognized, it has only recently become possible to measure the mechanical forces exerted by cells on their native microenvironments. Early investigations of cellular mechanics relied primarily on reductionist approaches carried out in culture such as laser ablation. This technique was initially used to sever small portions of the actomyosin network in order to provide insight into the force-generation machinery [38], and to demonstrate that local modifications in actomyosin contractility induce small changes in cell–cell and cell–ECM adhesions that lead to changes in cell shape, which can in turn induce tissue-scale outcomes [21]. Since then, laser ablation has been adapted to *in vivo* model systems, such as the *Drosophila* embryo, in order to provide a qualitative sense of how contractile forces are transmitted across developing tissues [39,40].

Even though laser ablation is a useful method that qualitatively reveals cellular tension in different tissue contexts, it does not provide quantitative information about cellular forces that might be at play during tissue development or disease progression. Recently, Campàs and colleagues developed ferrofluid oil droplets — or *microrheometers* — that can be injected into live tissues to measure the mechanical properties of the tissue surrounding the droplet, allowing one to infer the cellular forces within native tissues based on the deformation of the oil droplet (which has known shape and viscoelastic properties) [41,42].

Figure 2



Branching morphogenesis is regulated by mechanical forces imposed by the surrounding microenvironment. (a) Airway branching in the mouse lung is accompanied by localized smooth muscle differentiation at bifurcating tips [34] and (b) stratification of the epithelium during domain branching. (c) Modulating transmembrane pressure in a microfluidic device (d) alters the rate of branching of the murine airway epithelium [37\*\*].

This technique has been further developed to actively deform the droplet with the help of a magnetic field, and measure the mechanical response of the surrounding tissue. For instance, during tailbud elongation in the zebrafish embryo, which is used as a model system for vertebrate body axis elongation, the viscosity and stiffness of the tissue varied along the anterior–posterior axis, with the elongating

posterior region displaying lower tissue stiffness and increased fluidity, suggesting that the spatial variations in viscoelastic properties could be instrumental in tissue patterning. The use of ferrofluid oil droplets in tissues that have lost their normal architecture (e.g. tumors) could shed light on the mechanical changes that take place in the microenvironment during disease progression.

These methods, although disruptive, have contributed to our understanding of the cellular structures that sense and transmit mechanical information. Currently, non-invasive methods such as Förster resonance energy transfer (FRET)-based sensors, which do not deform the cell or tissue, are being used to study invertebrate development [43,44] and are in the process of being adapted for the study of force transmission in living vertebrate embryos. These sensors employ two fluorophores that are linked by a spring-like peptide that can be compressed or stretched reversibly by intracellular or extracellular forces depending on where the force sensor is anchored. Aside from providing insight into the role of mechanical forces in embryonic development, these techniques may also promote the engineering of biomaterials that effectively mimic various *in vivo* mechanical microenvironments, and offer new avenues to investigate the role of mechanics in disease progression.

### Towards building organs from scratch

Epithelial folding is a highly complex yet reproducible process *in vivo*. The ability to recapitulate epithelial folding and organ development in culture would greatly accelerate understanding of the underlying mechanisms and screening of therapeutics. Current efforts in 3D tissue culture models are directed towards recapitulating the complexity of mammalian organogenesis by engineering stem cells, constructing biomimetic materials, and directing tissue architecture in novel culture systems [45]. Over the past century, these efforts have evolved from culturing tissue fragments [46–48] and generating organ-like structures in suspension from dissociated cells [49], to recognizing the role of the ECM in orchestrating tissue morphogenesis [50–52]. 3D collagen or laminin-rich cultures [53,54], micropatterning approaches [3], and more recently, advances in stem cell engineering have paved the way for organoid models of epithelial tissues [55].

Programming of stem cells requires profound understanding of how single cells can be ordered to assemble into epithelial structures, as in the establishment of the long-term culture of multipotent Lgr5<sup>+</sup> stem cells that gave rise to ‘mini guts’ [56]. Advances in stem cell engineering have paved the way for organoid models of epithelial tissues. Recently, the directed differentiation of human pluripotent stem cells into progenitors of the ureteric epithelium or the metanephric mesenchyme that develop into collecting ducts and nephrons, respectively, has led to human kidney organoid cultures that contain renal tubules [57]. These organoid models have several advantages, including accessibility through high-resolution imaging, temporal control, and genetic manipulation, and are promising models of human disease [58,59]. However, organoids are not perfect in the sense that they can only recapitulate certain stages of development and are often difficult to reproduce [60]. In order to induce the development of more refined structures, the characteristics of a microenvironment that can support the differentiation and self-organization of stem cells into organoids need to be determined.

The spatiotemporal distribution of microenvironmental signals determines how these cues will be received and interpreted by cells. For example, intestinal stem cell survival, proliferation, and self-organization can be modulated by synthetic hydrogel networks with tunable ECM stiffness [61], a mechanical property that can now be controlled with spatial precision by modulating the cross-linking of polyethylene glycol (PEG) hydrogels [62]. Such biomimetic scaffolds were shown to allow for intestinal stem cell survival and organoid formation [61]. However, inducing tissue folding *in vitro* to achieve the complexity of mammalian organogenesis still poses a significant challenge. Recently, inspired by the local strain differences that arise between the folding epithelium and the underlying tissue in many morphogenetic processes, Hughes *et al.* devised an approach to pattern fibroblasts on ECM-based gels, and observed that these cells, by pulling on the surrounding ECM fibers, created local strains at the epithelial–mesenchymal interface that led to epithelial folding at precise locations similar to the patterning of the mouse gut [63<sup>\*\*</sup>]. Endeavors aimed at mimicking cell–cell and cell–ECM interactions in native cellular microenvironments and instructing self-organization of epithelial sheets *in vitro* could guide future efforts towards fabrication of tissues that have physiological function.

### Conclusions

Cells interact with their surrounding microenvironment in a reciprocal manner, and these interactions are often inhomogeneous, anisotropic, and transient. The spatiotemporal regulation of how mechanical and biochemical signals are perceived and transmitted by cells sculpts epithelial sheets into tissues and organs with unique bends, folds, and curves to accommodate their function. These complex interactions can be partially recapitulated using 3D models, which are becoming more sophisticated with the advent of organoids and engineered hydrogels.

### Conflict of interest statement

Nothing declared.

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